L2: Entry 6 of 10 File: USPT May 31, 1994

DOCUMENT-IDENTIFIER: US 5316912 A
TITLE: Polyacrylic hydrazides and their applications as glycoprotein reagents

Abstract Text (1):
A method for detecting a glycoprotein using a solid support is disclosed where the glycoprotein is oxidized by periodate, polyacrylic polyhydrazide which is a copolymer having repeating units possessing a hydrazide group and repeating units possessing hydroxyl groups is coupled to the oxidized glycoprotein and a glycoenzyme or radioactive compound containing aldehyde groups or activated ketone groups is coupled to the polyacrylic polyhydrazide which allows for detection of the glycoprotein. The glycoprotein may be directly attached to the solid support or may be bound to an antigen which is immobilized on the solid support.

Brief Summary Text (13):
We have found that suitable polyacrylic hydrazides are conveniently prepared through co-polymerization of a neutral, hydrophilic monomer and a monomer containing an activated ester group. Neutral monomers include acrylamide, N-acryloyl-tris-(hydroxymethyl)-aminomethane and N-acryloyl-2-amino-2-deoxy-D-glucitol, whereas N-acryloxysuccinimide was used as an activated monomer. The reaction of hydrazine with the copolymer produced the desired polyacrylic hydrazides. Such polyhydrazides couple readily to periodate oxidized glycoproteins, for example to horse radish peroxidase (HRP) and immunoglobulins. Detection of glycoproteins on solid support, such as membranes consists mainly of periodate oxidation of these glycoproteins and polyhydrazide mediated coupling of HRP to the modified glycoproteins. Staining on HRP activity then locates the glycoproteins. The coupling of HRP is carried out through the aldehyde groups generated in the carbohydrate chains of this glycoenzyme. The detection method provided in this invention is specific for glycoproteins and its sensitivity for highly glycosylated proteins is comparable to the sensitivity of Aurodye (Janssen) staining of the blots. Detection of antigens is performed through the antibodies which have been oxidized by periodate. The polyhydrazides serve as a bridge between the oxidized sugar chains of an antibody and the oxidized sugar chains of an glycoenzyme, such as horse radish peroxidase (HRP). This approach offers several advantages, such as the attachment of a label to the inert sugar chains of antibodies and the possibility to avoid the use of secondary antibodies.

Detailed Description Text (15):
a. The "two incubations protocol". FIG. 1 shows a scheme for detection of glycoproteins. A glycoprotein is immobilized on a solid support (membrane) and oxidized by periodate (panel 1). The oxidized glycoprotein is reacted with a polyhydrazide (panel 2) or another bifunction or multifunction hydrazide. Some of the hydrazide groups react with the aldehydes generated in the sugar chains of the immobilized glycoprotein, and some remain free. The free hydrazide groups are reacted with the aldehyde groups generated in the carbohydrate chains of a soluble glycoenzyme, such as peroxidase (panel 3). The bound enzyme (panel 4), and through it the immobilized glycoprotein, is located with a suitable substrate which is usually converted to a colored product by the enzyme.

CLAIMS:

1. A method for detection of an oxidized glycoprotein comprising the steps of:

a) binding a glycoprotein to a solid support;

b) oxidizing the bound glycoprotein with periodate to form an oxidized glycoprotein wherein aldehyde or ketone groups are formed by oxidizing carbohydrate residues of said glycoprotein;

c) reacting said oxidized glycoprotein with a polyacrylic polyhydrazide wherein said polyacrylic polyhydrazide binds to said oxidized glycoprotein, and wherein said polyacrylic polyhydrazide is a copolymer comprising repeating units possessing a hydrazide group and repeating units possessing hydroxyl groups;

d) reacting a label with said polyacrylic polyhydrazide, wherein said label contains aldehyde groups or activated ester groups which bind to said polyacrylic polyhydrazide through hydrazide groups on said polyacrylic polyhydrazide; and

e) detecting said oxidized glycoprotein bound to said support through detection of said polyacrylic polyhydrazide having said label bound thereto.

13. A method for detection of an oxidized glycoprotein comprising the steps of:

a) oxidizing a glycoprotein with periodate to form an oxidized glycoprotein wherein aldehyde or ketone groups are formed by oxidizing carbohydrate residues of said glycoprotein;

b) binding said oxidized glycoprotein to a solid support;

c) reacting said oxidized glycoprotein with a polyacrylic polyhydrazide wherein said polyacrylic polyhydrazide binds to said oxidized glycoprotein, and wherein said polyacrylic polyhydrazide is a copolymer comprising repeating units possessing a hydrazide group and repeating units possessing hydroxyl groups;

d) reacting a label with said polyacrylic polyhydrazide, wherein said label contains aldehyde groups or activated ester groups which bind to said polyacrylic polyhydrazide through hydrazide groups on said polyacrylic polyhydrazide; and

e) detecting said oxidized glycoprotein bound to said support through detection of said polyacrylic polyhydrazide having said label bound thereto.